Whole Cell Panning of Libraries by
Magnetically-Activated Cell Sorting (MACS)
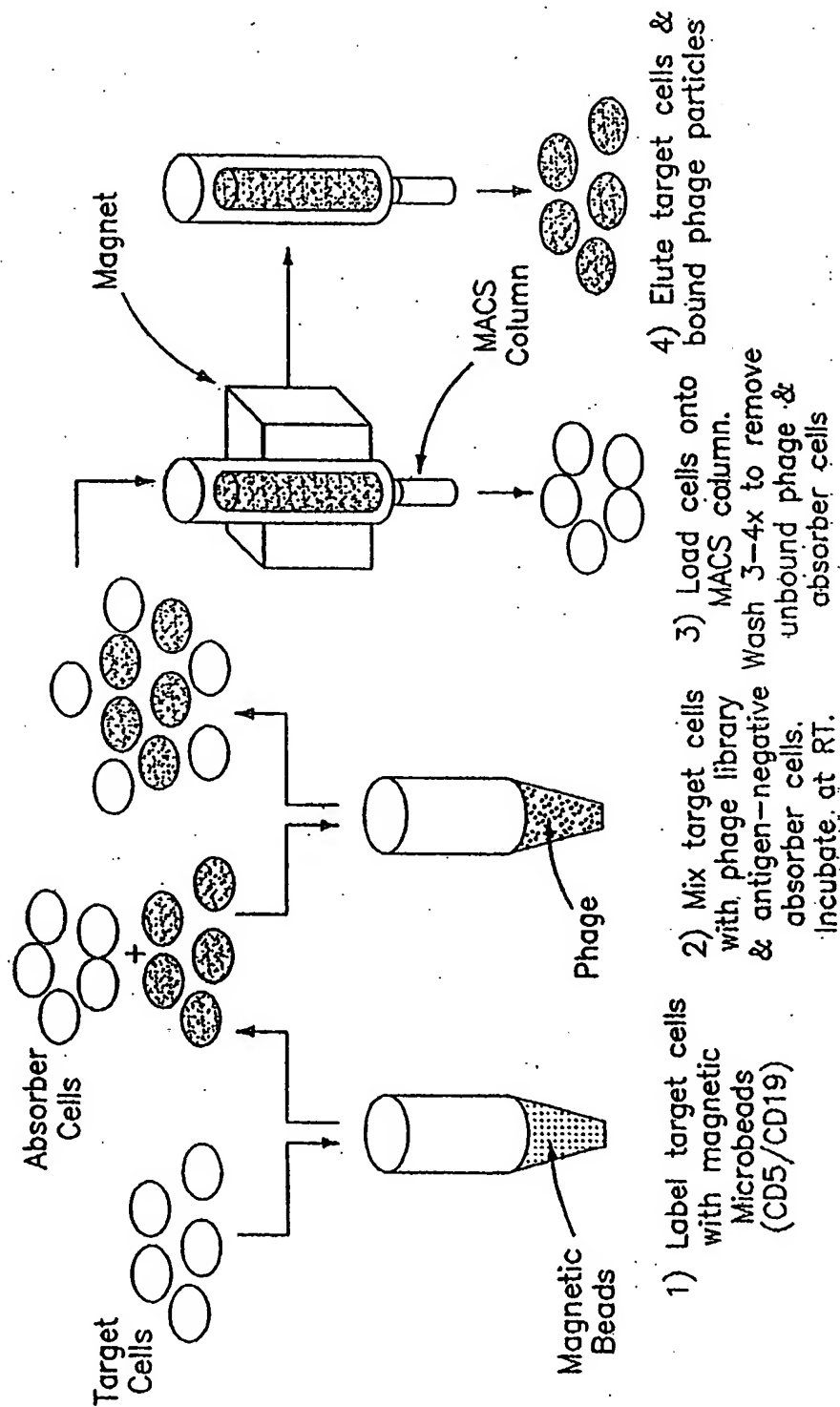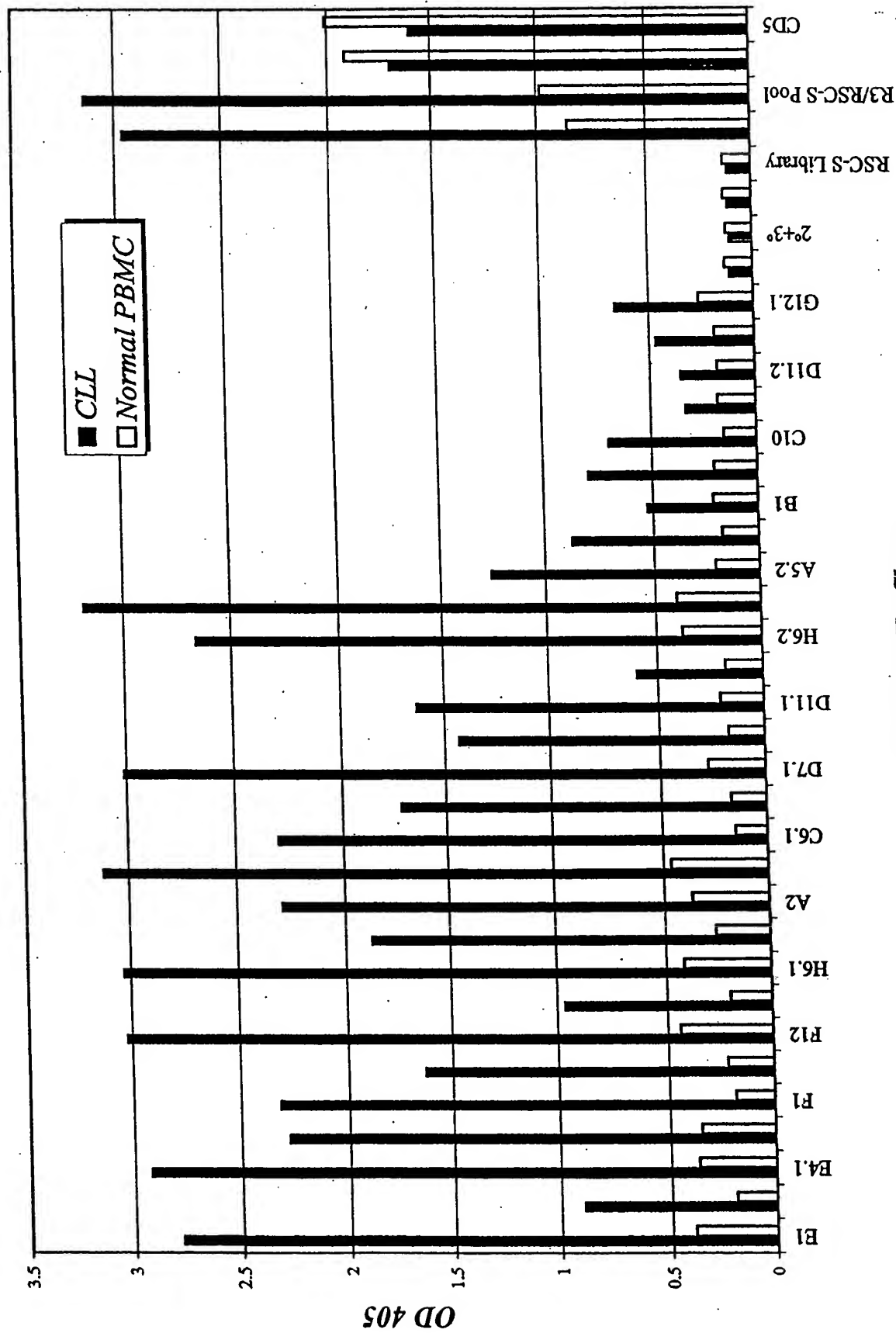
Target
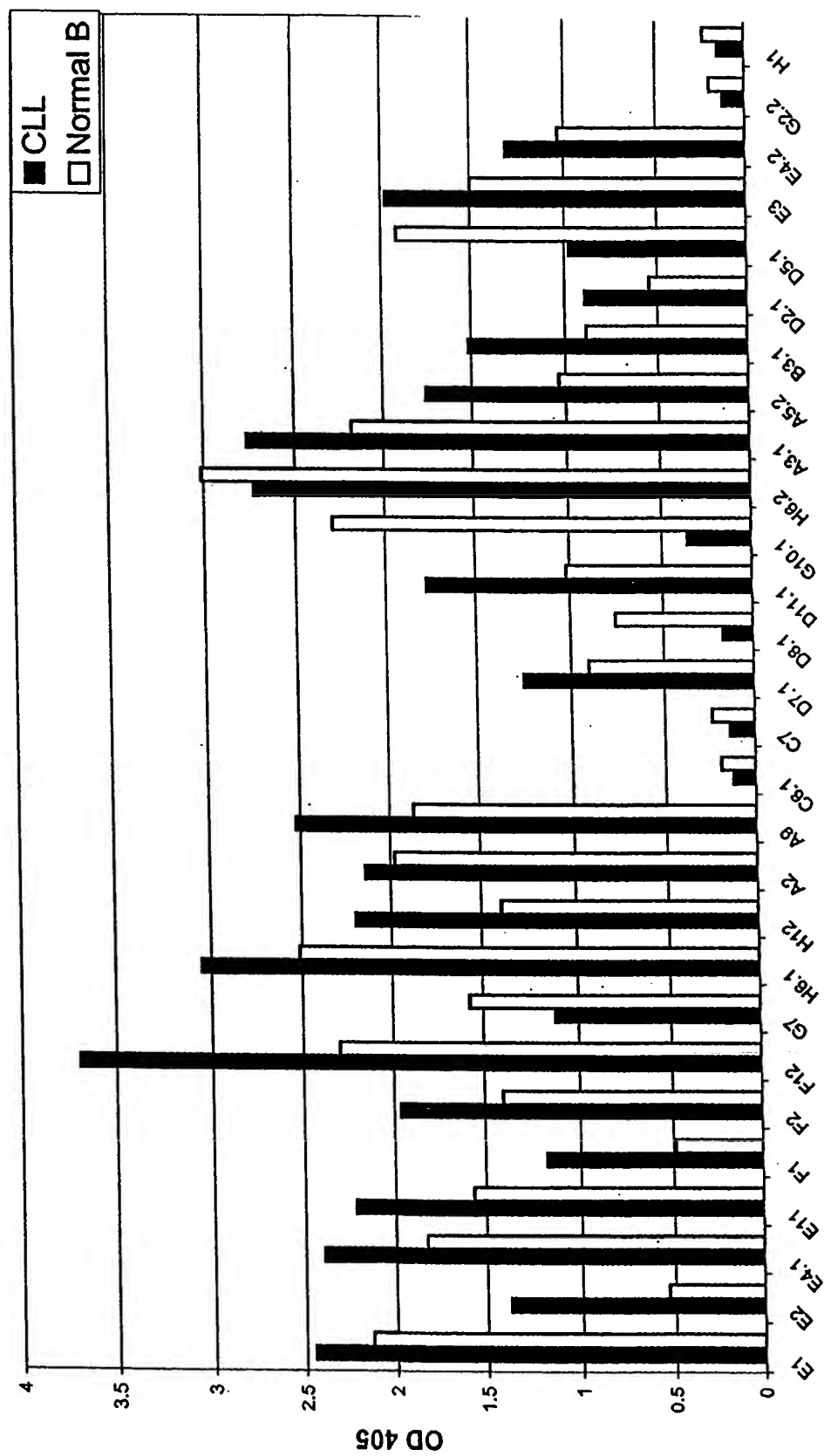Cells

Absorber
Cells

+

Magnetic
Beads

Phage

Magnet

MACS
Column

1) Label target cells
with magnetic
Microbeads
(CD5/CD19)

2) Mix target cells
with phage library
& antigen-negative
absorber cells.
Incubate at RT.

3) Load cells onto
MACS column.
Wash 3-4x to remove
unbound phage &
absorber cells

4) Elute target cells &
bound phage particles

Fig. 1

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Fig 2. CELL ELISA-5/5/00

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Fig 3a. CELL ELISA 4/22/00

Legend: ■ CLL □ Normal B

X-axis (Rabbit scFv Clone): E1, B2, E4.1, E11, F1, F2, F12, G7, H6.1, H12, A2, A9, C8.1, C7, D7.1, D8.1, D11.1, G10.1, H8.2, A3.1, A5.2, B3.1, D2.1, D5.1, E3, E4.2, G2.2, H1

Y-axis (OD 405): 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4

Fig 3b. CELL ELISA 4/22/00

Legend: ■ CLL, □ Normal B

X-axis (Rabbit scFv Clone): H6.3, A8, B12.1, C12, D1.1, D5.2, D8.2, F10, A1.1, G8, B1, B4.2, C10, D4.2, D11.2, G1.2, D2.2, G12.1, R3/RSC5 Pool, RSC1 Library, R3/RSC1 Pool, R4/RSC1 Pool, R5/RSC1 Pool, no antibody, 2°+3°, CD5, CD19

Y-axis (OD 405): 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4

Fig 4a. CELL ELISA 5/21/00

Legend: RE, MP, DJ

Y-axis: OD 405 (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5)

X-axis (scFv Clone): E3, E2, E41, E11, F1, F2, F12, G7, H81, H12, A2, A9, C81, C7, D71, D81, D11.1, G10.1, H6.2, A3.1, A5.2, B3.1, D2.1, D5.1, E3, E4.2, G2.2, H1, H6.3, A8, B12.1, C12, D1.1, D5.2, D8.2, F10

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Fig 4b. CELL ELISA 5/21/00

scFv Clone

OD 405

RE
MP
DJ

Fig 5a. CELL ELISA 8/19/00

Legend: CLL, RAMOS, RL, TF1

X-axis: Rabbit scFv Clone — E1, E2, E4.1, E11, F1, F2, F12, G7, H6.1, H12, A2, A9, D7.1, D11.1, G10.1, H6.2, A3.1, A5.2, B3.1, D2.1, D5.1, E3

Y-axis: OD 405 — 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5
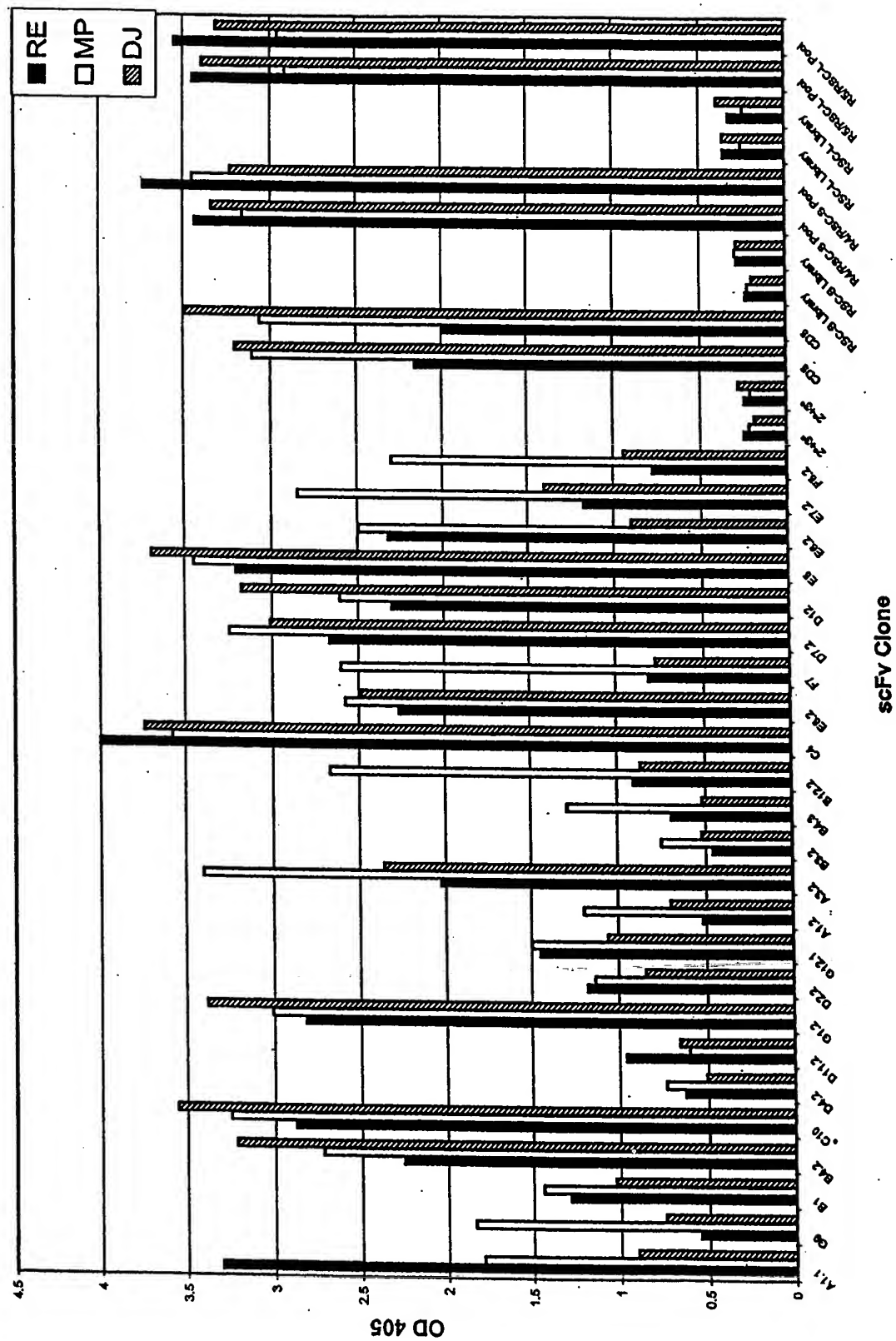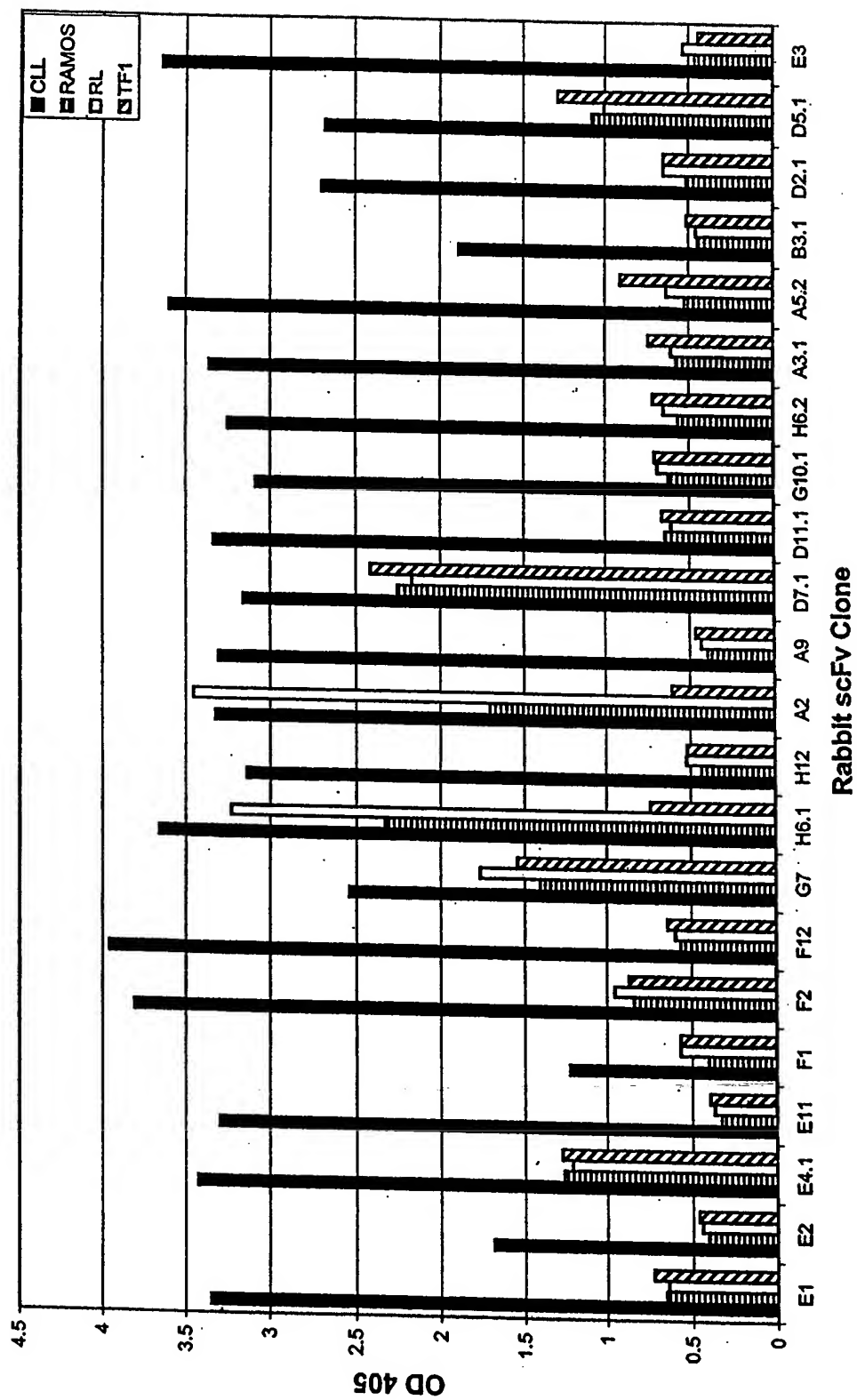
Fig 5b. CELL ELISA 8/19/00

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Fig 6a. CELL ELISA 9/15/00

Legend: ■ CLL □ CLL-AAT ▨ TF-1

X-axis (Rabbit scFv Clone): E1, E2, E4.1, E11, F1, F2, F12, G7, H6.1, H12, A2, A9, D7.1, D11.1 G10.1, H6.2, A3.1, A5.2, B3.1, D2.1, D5.1

Y-axis (OD 405): 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5

Fig 6b. CELL ELISA 9/15/00

Legend: CLL, CLL-AAT, TF-1

Y-axis: OD 405

X-axis (Rabbit scFv Clone): E3, E4.2, A8, B12.1, C12, D1.1, D5.2, D8.2, F10, A1.1, G9, B1, B4.2, C10, D4.2, D11.2, G1.2, D2.2, G12.1, A1.2, A3.2
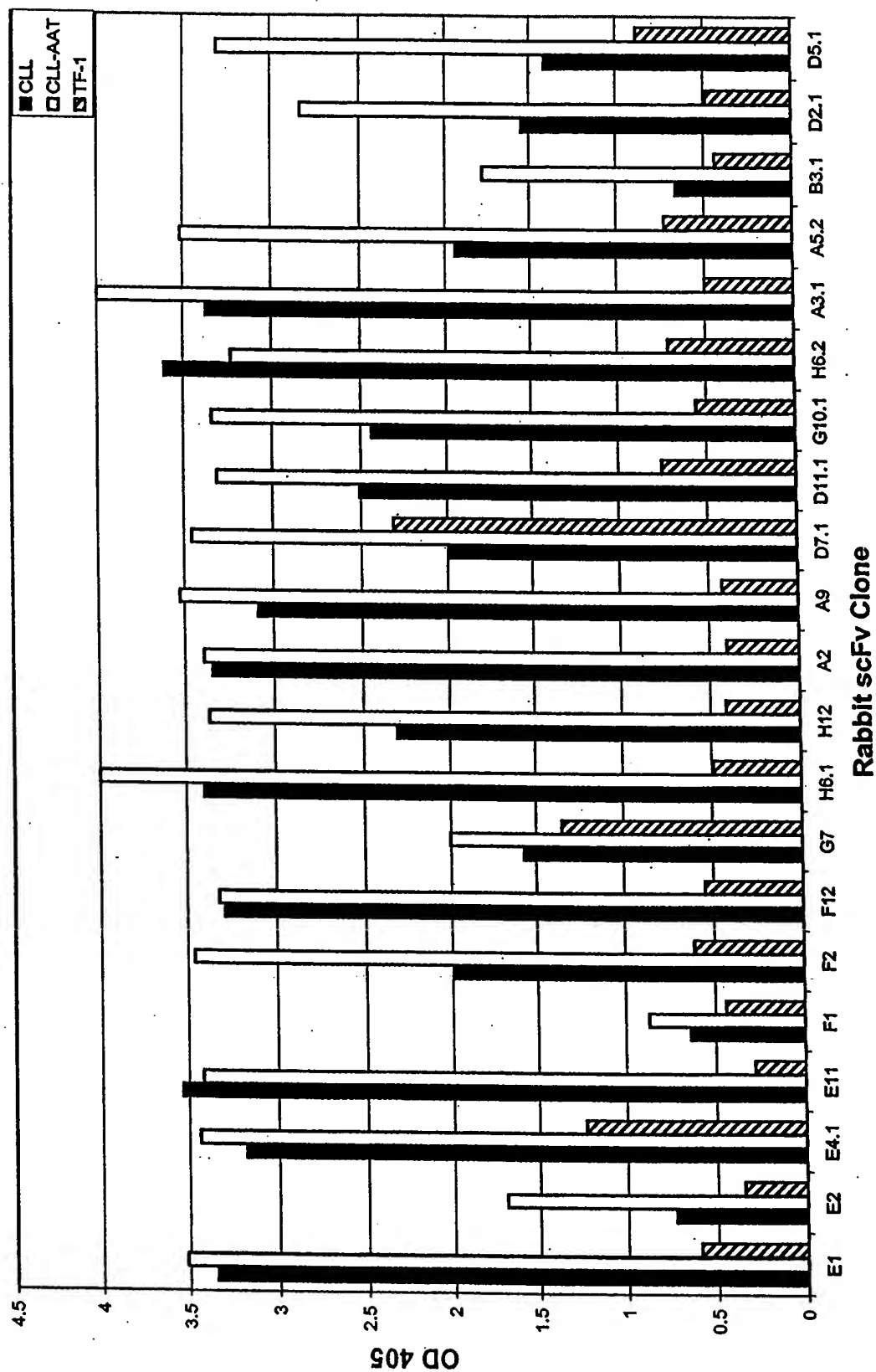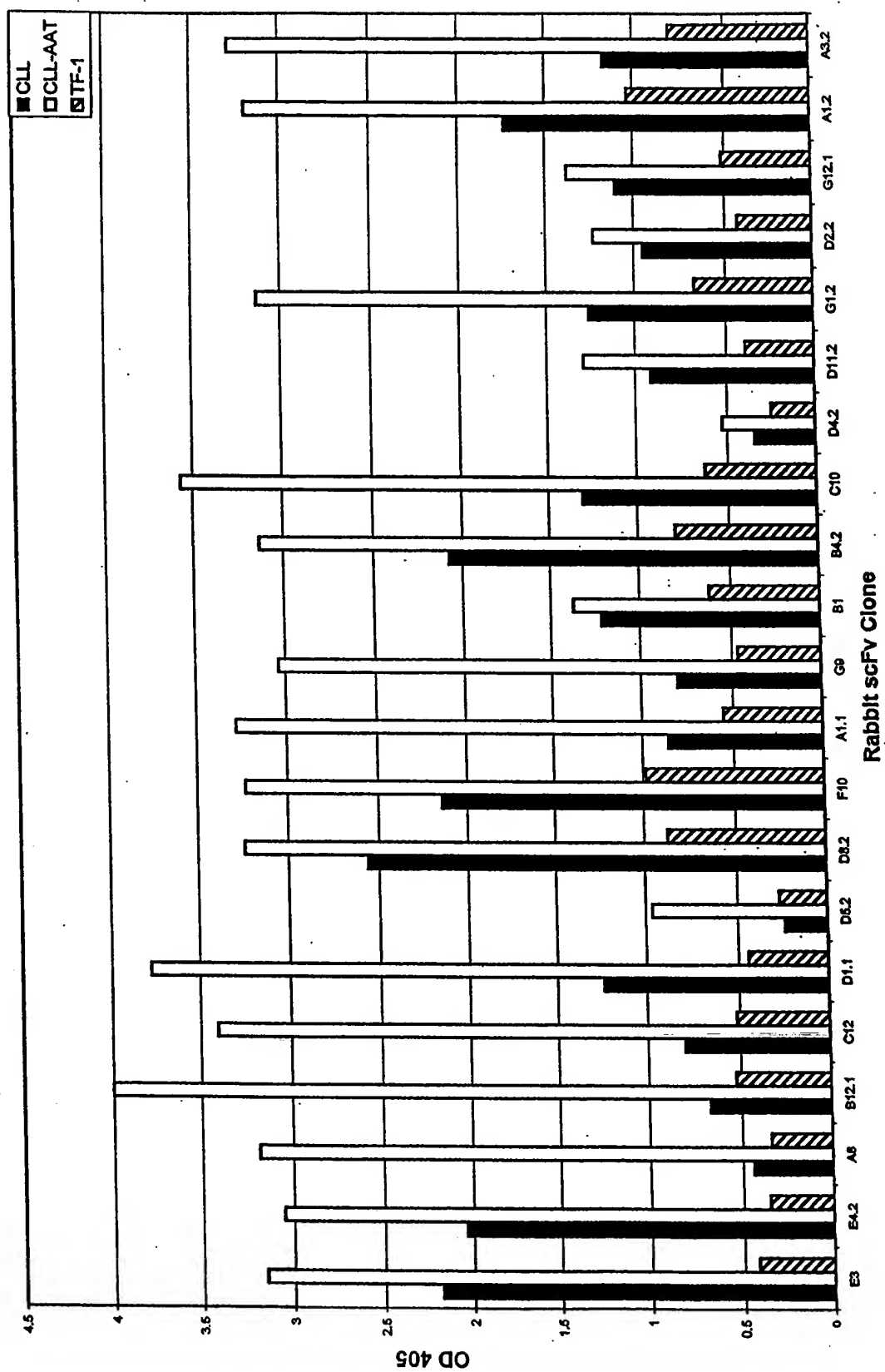
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Fig 6c. CELL ELISA 9/15/00

Legend:
- CLL
- CLL-AAT
- TF-1

X-axis (Rabbit scFv Clone): B3.2, B4.3, B12.2, C4, E8.2, F7, D7.2, D12, E5, E8.3, E7.2, F5.2, UNSTAINED, 2°+3°, RSC-S LIBRARY, R3/RSC-S, CD5, CD19, CD20, CD23, CD38
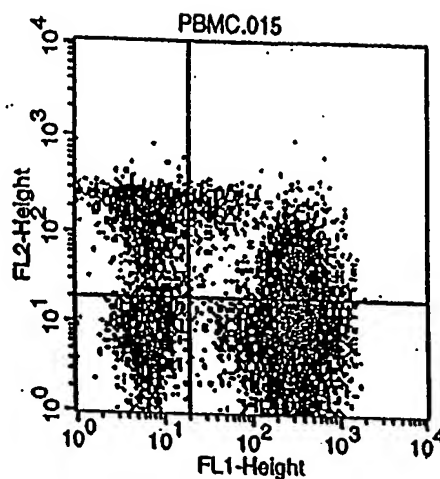
Y-axis (OD 405): 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4
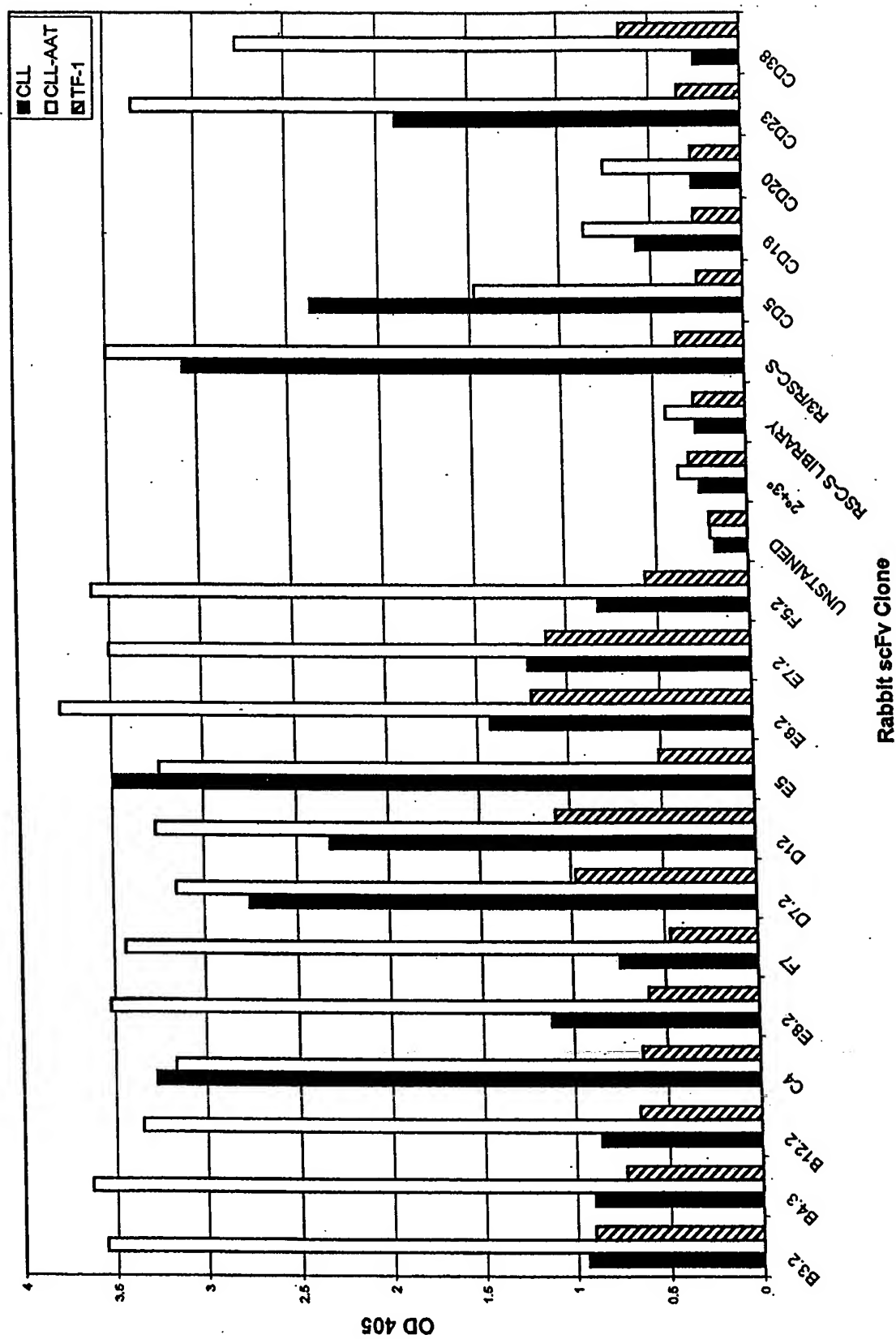
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Figure 7

FL2: scFv-9/HA-biotin/SA-PE
FL1: CD5-FITC
FL3: CD19-PerCP

PBMC.015 (SSC-Height vs FSC-Height)

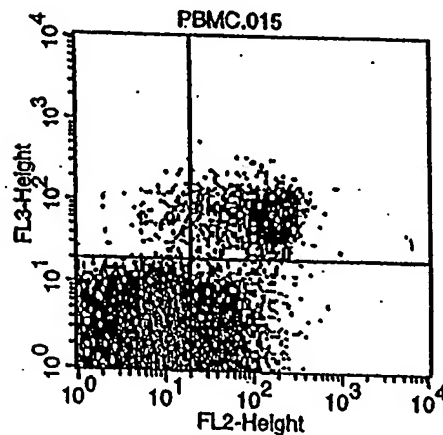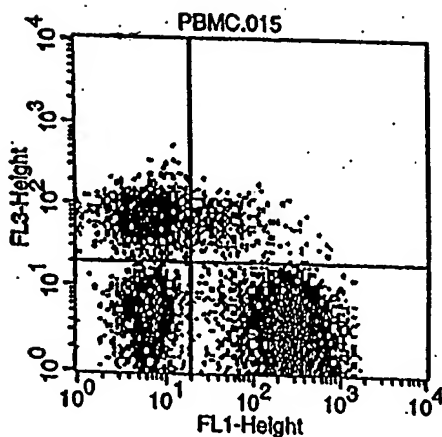PBMC.015 (FL2-Height vs FL1-Height)
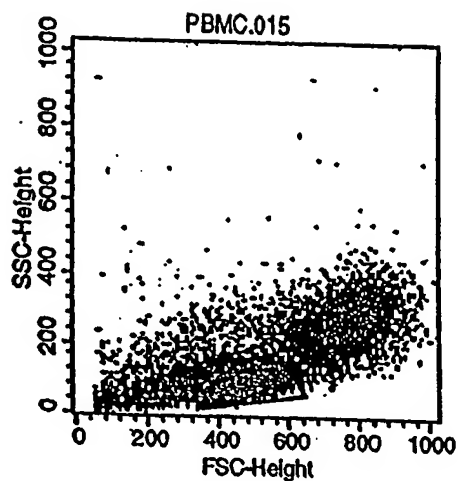
File: PBMC.015
X Parameter: FL1-H FL1-Height (Log)
Y Parameter: FL2-H FL2-Height (Log)

Quad	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
UL	1881	9.40	5.84	6.45	118.74
UR	4368	21.84	13.56	266.89	45.49
LL	2831	14.16	8.79	6.65	7.40
LR	10920	54.60	33.90	282.52	5.72

PBMC.015 (FL3-Height vs FL1-Height)

File: PBMC.015
X Parameter: FL1-H FL1-Height (Log)
Y Parameter: FL3-H FL3-Height (Log)

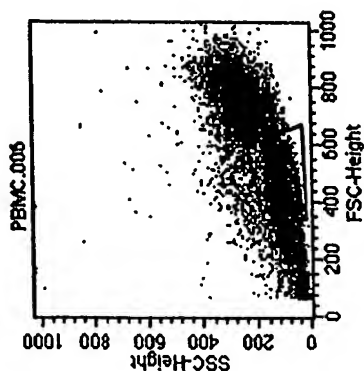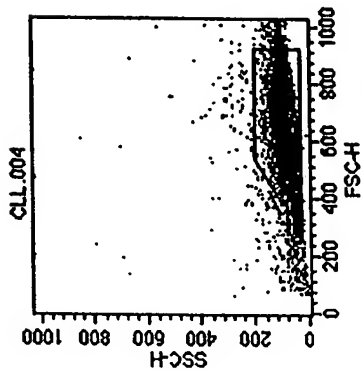| Quad	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
UL	1874	9.37	5.82	6.55	65.56
UR	409	2.04	1.27	50.57	55.81
LL	2838	14.19	8.81	6.57	4.19
LR	14879	74.39	46.19	291.30	2.17

PBMC.015 (FL3-Height vs FL2-Height)

File: PBMC.015
X Parameter: FL2-H FL2-Height (Log)
Y Parameter: FL3-H FL3-Height (Log)

Quad	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
UL	171	0.85	0.53	10.16	54.88
UR	2112	10.56	6.56	137.20	64.47
LL	13744	68.72	42.67	6.08	2.52
LR	3973	19.86	12.33	41.31	2.08

Figure 8a

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Figure 8b

scFv-3
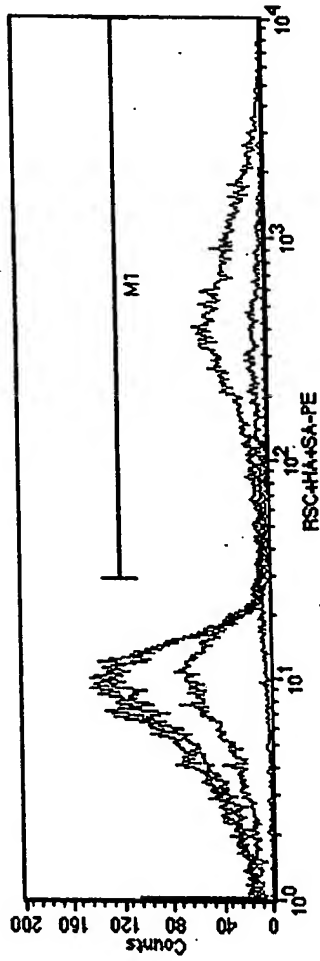
Key	Name	Parameter	Gate
—	CLL.004	FL2-H	G3
—	CLL.009	FL2-H	G3
—	PBMC.006	FL2-H	G1
—	PBMC.010	FL2-H	G1

Counts vs RSC+HA+SA-PE (M1)

* Numerator Histogram *
File: PBMC.010
* Denominator Histogram *
File: PBMC.006

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9991	20386	100.12	64.95	34.40	8.94	351.33	8.35	1
M1	28, 9991	1953	9.59	6.22	281.98	176.72	103.08	201.65	271

* Numerator Histogram *
File: CLL.009
* Denominator Histogram *
File: CLL.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9991	10258	99.82	86.73	587.42	545.56	104.91	392.42	385
M1	28, 9991	9768	95.24	82.76	614.87	403.58	100.46	414.18	385

Figure 8c

scFv-9
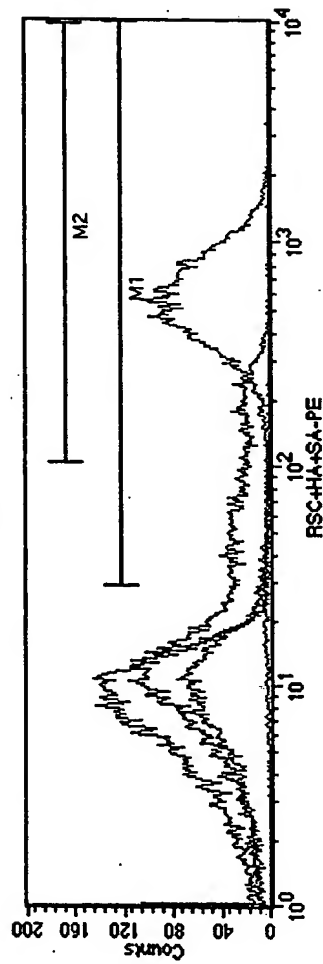
Key	Name	Parameter	Gate
—	CLL.004	FL2-H	G3
—	CLL.018	FL2-H	G3
—	PBMC.006	FL2-H	G1
—	PBMC.014	FL2-H	G1

* Numerator Histogram '
File: CLL.018

* Denominator Histogram '
File: CLL.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9911	10272	99.95	86.85	568.16	481.42	48.32	528.02	528
M1	28, 9911	10073	98.01	85.17	579.08	515.60	45.93	532.80	528
M2	103, 9911	9924	96.57	83.91	587.05	534.82	44.25	537.61	528

* Numerator Histogram '
File: PBMC.014

* Denominator Histogram '
File: PBMC.008

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9911	20356	100.16	64.63	34.38	15.08	173.46	12.30	1
M1	28, 9911	5229	25.74	16.60	103.57	80.28	82.38	71.05	38
M2	103, 9911	1893	9.32	6.01	190.40	179.03	45.79	177.83	168

Figure 9a Table 1. Summary of CLL scFv Clones

| Pool	Clone	CLL	Primary B	CLL-AAT	RL (NHL)	Ramos (Burkitt's)	TF1	Patient-Specific	Expression Lost	Fingerprint
R3/RSC-S CLL-TF1	E1	++	++	++	-	-	-			1
	E2	+	-	+	-	-	-			2
	E4.1	++	+	++	±	±	±			3
	E11	++	+	++	-	-	-			4
	F1	+	-	+	-	-	-			5
	F2	++	+	++	-	-	-			6
	F12	++	+	++	-	-	-			7
	G7	+	+	+	+	+	+			8
	H6.1	++	+	++	+	+	+			7
	H12	++	+	++	-	-	-			9
	A2	++	++	++	+	+	-			10
	A9	++	+	++	-	-	-			11
	C6.1	-	-	nd	nd	nd	nd	+		12
	C7	-	-	nd	nd	nd	nd	+		13
	D7.1	+	+	+	+	+	+			14
	D8.1	-	-	nd	nd	nd	nd	+?		15
	D11.1	++	+	++	-	-				16
	G10.1	+	+·	+	-	-				17
	H6.2	++	++	++	-	-	-			18
	A3.1	++	+	++	-	-	-			19
	A5.2	++	+	++	-	-	-			20
	B3.1	+	+	+	-	-	-			9
	D2.1	+	+	+	-	-	-			21
	D5.1	+	+	+	±	±	±			22
	E3	+	+	+	-	-	-			23
	E4.2	+	+	+	-	-	-			24
	G2.2	-	-	nd	nd	nd	nd	+		25
	H1	-	-	nd	nd	nd	nd	+		26
	H6.3	-	-	nd	nd	nd	nd	+		27
R3/RSC-L CLL-TF1	A8	-	+	+	nd	nd	nd	+?		28
	B12.1	+	+	++	nd	nd	nd			29
	C12	++	+	++	+	+	±			30
	D1.1	+	+	+	nd	nd	nd			31
	D5.2	-	+	+	nd	nd	nd	+?		32
	D8.2	++	+	++	+	+	±	+?	+	33
	F10	++	+	++	+	+	±			34
	A1.1	+	+	++	nd	nd	nd			(nd)
	G9	±?	+	++	nd	nd	nd	+?		(nd)
R5/RSC-L CLL-B	B1	+	+	+	nd	nd	nd			35
	B4.2	++	+	++	nd	nd	nd			36
	C10	++	+	++	nd	nd	nd			37
	D4.2	-			nd	nd	nd	+?	+	38
	D11.2	±?	-	+	nd	nd	nd	+?		39
	G1.2	++	+	++	-	-	-			37
	D2.2	+	+	+	nd	nd	nd			40
	G12.1	+			+	+	-			41
R4/RSC-S CLL-B	A1.2	±?	nd	++	nd	nd	nd	+?		42
	A3.2	+	nd	++	+	+	±			43
	B3.2	-	nd	+	nd	nd	nd	+		44
	B4.3	-	nd	++	nd	nd	nd	+		45
	B12.2	+	nd	++	+	+	±			46
	C4	++	nd	++	-	-	-			47
	E8.2	++	nd	++	-	-	-			48
	F7	+	nd	++	+	+	-			46
	D7.2	++	nd	++	+	+	±			49
	D12	++	nd	++	+	+	+			50
	E5	++	nd	++	+	+	-			51
	E6.2	+	nd	++	+	+	+			52
	E7.2	+	·nd	++	+	+	+			53
	F5.2	+	nd	++	+	+				54

Legend:
- CLL + Primary B Cells
- CLL Cells
- CLL+ All B Cells
- CLL + All B Cells + TF1dim
- CLL + All B Cells + TF1bright
- patient-specific or lost expression
- not fully characterized

FIGURE 9B

Table 1. CDR Sequences of CLL Specific Rabbit scFv Antibodies

SEQ	CLONE	LC-CDR1	LC-CDR2	LC-CDR3	HC-CDR1	HC-CDR2	HC-CDR3	Expression Pattern: CLL	B	RL	Ramos	TF-1	Ag	Linker
1	A2c	TLSTGYSVGSYVIA	HSEEAKHGQS	ATARGSGSFHVV	NYAMT	IISSNGGA--DYASWAK	DDEGYDDYGDRNGYYTL	+	+	++	+	-		S
2	G12.1c	QASSIRN---YLA	GASNL---ES	QSGYTSA---GLT	SYGLS	YFDVFGRT-YTATVD	DRIYVSSVG---YAFNL	+	+	+	+	-	CD19	L
17	B4.2a	QASESRN---YLA	GASNL---ES	QSGYTSA---GLT	TYGVS	YNDPIFGRT-YTATNVN	DRAXASSSG---YXXXX	++	nd	+	+	-		L
3	E1c	QASESISN---WLA	PASTL---AS	QSGYTSA---GVT	SNAMG	IISSSGGT--YTASWAK	DMIAAGRS----YGLDL	++	+	+	+	-	CD23	S
18	F2d	QASESISN---YLA	GASNL---AS	QSGYTSA---GLT	TYAMG	IISSSGST--YTASWAK	DMIAAGRS----YGLDL	++	+	±	-	-		S
4	E5e	QASQNIYS---NLA	LAPTL---AS	QXGDYSSSSYGYG	SSDWIC	CIYTGSSSTYYDASWAK	RIYGDWG--------NL	±	nd	-	nd	-		S
5	H6.2b	QASQSVRN---LLA	GASNL---ES	QSGYTSP----GVT	SDVIS	YIYTDDGSP-DYASWN	DAAYAGYGW----IFNL	++	++	+	+	-		S
19	G10.1	QASESINN---YLA	GASNL---ES	QSGYTSG---GAT	SDVIS	YIYTDGST-DYASWVN	DAAYAGYGW----IFNL	++	+	+	+	-		S
6	D11.1c	LASENVYS---AVA	GASNL---ES	Q-GYSYPT	TYAMG	SIYASRSP--YYASWAK	GDAGSIP-----YFKL	++	+	+	+	-	CD23	S
20	A5.2c	LASENVYG---AVA	GASNL---ES	Q-GYSYP-T	TYAMG	SIYASRSP--YYASWAK	GDAGSIP-----YFKL	+	+	+	+	-		S
7	F1d	QASQSVRN---LLA	GASNL---ES	AGYKSSSD-GIA	SNAMT	TIIYGDNT--YTANWAK	GNV--------FSDL	++	+	-	-	nd		S
8	F1e	QASQSISN---LLA	GASNL---ES	QSGYTSA--GHLT	DFAMS	VVYAGTRGDTYTANWAK	GLT---------YYPL	++	nd	nd	-	nd		S
21	E4.2	LASENVAS---TVS	GASNL---ES	LGGYGYSYT-GLT	SYGWN	YIDPDYGST-YTASWVN	GAYSGYPS----YFNL	+	+	-	-	nd		L
9.	E2c	TLSTGYSVGEYFVV	HTDDIKHGQS	ALARGTESSFHVV	SYGWN	YIDPDYGST-YTASWVN	GAYSGYPS----YFNL	++	+	+	-	nd		S
	A9c	TLSTGYSVGEYFDV	HTDDIKHGQS	ALARGTESSFHVV	SYGWN	ITYPSGNY--YTASWAK	GNA--------FFNL	++	+	-	-	-		S
10	E11e	TLRTGYSVGEYFDV	HTDDIKHGQS	ATGRGSGSSAGVV	SNAMS	ITYPSGNY--YYASWAK	GNA--------YSDL	++	+	-	-	-		L
11	A1.1	LASEDIYS---GLS	GASNL---ES	LGGYYTSST-GTA	TNAIS	YSSYGNRA--HYTATAK	DQPIIGANGDYGLAYGYRLDL	+	+	nd	nd	nd		L
12	F5.2	QASQSVSN---LLA	GASNL---ES	QSGYTSA--GALT	SNAMS	IIIGSGT--YTATAK	DQPIIGANGDYGLAYGYRLDL	nd	nd	+	+	nd		L
22	F10b	QASQSVRN---LLA	PASTL---AS	QSGYTRA--GDLT	SYIMS	IISSSGS--YTATAK	DQPIIDAAYGDYGIATGYRLDL	nd	nd	nd	nd	nd		L
23	F7a	QASQSVSN---LLA	GASNL---ES	QSGYTSA--GLT	SYIMS	IISSGSA--YTATAK	DQPIITGDYGGDYGIATGYRLDL	nd	nd	nd	nd	nd		L
13	F6b	QASQSVSN---LLA	GASNL---ES	QSGYTSA--GLT	SNAIS	IIVGSGTY--YTADAK	DQPIITAGYGY--AYGYRLDL	nd	nd	nd	nd	nd		L
24	C12b	QASQSVSN---LLA	GASNL---ES	QSGYTSA--GALT	SNAIS	IIVGSGT--YTADHAK	DQPIITAGYGY--ACGYRLDL	nd	nd	nd	nd	nd		L
14	D2.1b	QASQSVNN---LLA	GASNL---ES	QSGYTSA---GLT	TNAMS	TITYGRA--YTADAK	GNT--------YSDL	+	+	nd	nd	nd		L
25	D1.1	QASEDIES---YLA	LASKL---AS	QSRANSY---GWT	SNAM9	TITYGRA--YYASWAK	GNT--------YSDL	nd	nd	nd	nd	nd		L
15	D2.2a	QSSQSIAGA--YLS	LASRL---AS	AAQYSGN---IYT	SSYWIC	CIYTGSNGSTYYASWAK	AYIYGGYG---FFDL	nd	nd	nd	nd	nd		L
16	D2.2b	LASENVYG---AVA	GASNL---ES	Q-GYSYP-T	NYGVN	YIDPYFGST-YYASWVN	ESFYY------GMDL	nd	nd	nd	nd	nd		S

SEQ: sequence designation
CLONE: designation of representative clone for sequence.
LC: Ig light chain
HC: Ig heavy chain
CDR: complementarity determining region
Expression pattern: binding of scFv antibodies to primary human cells and cell lines as determined by whole cell ELISA assay
CLL: chronic lymphocytic leukemias (primary tumors and CLL-AAT cell line)
B: normal, primary human B lymphocytes
RL: non-Hodgkin's lymphoma cell line
Ramos: Burkitt's lymphoma cell line
TF-1: human erythroleukemia cell line
Ag: antigen recognized by scFv antibody (determined by immunoprecipitation and mass spectrometry)
Linker: type of linker sequence between VL and VH regions. S, short linker; L, long linker

FIGURE 10

Table 2. Mean fluorescent intensities of B-CLL cells and normal PBMC labeled with scFv antibodies

Donor	Antibody and CLL/PBMC Ratio: scFv-2	ratio	scFv-3	ratio	scFv-6	ratio	scFv-9	ratio
CLL(ML)	590	0.83	398	2.2	284	2.1	511	6.4
PBMC-1	715		181		137		80	
CLL(JR)	311	0.85	207	2.4	nd	nd	117	1.7
PBMC-2	368		87		nd		67	
CLL(HTS)	219	0.69	173	1.6	nd	nd	176	3.6
PBMC-3	317		106		nd		49	
CLL(RE)	305	0.59	360	3	nd	nd	142	1.7
PBMC-4	513		121		nd		81	
CLL(GB)	262	0.47	387	1.8	nd	nd	163	1.5
PBMC-5	563		212		nd		106	

Primary PBMC from five patients diagnosed with CLL and five normal donors were analyzed by flow cytometry. The geometric mean fluorescent intensities were determined for cells stained with four different scFv antibodies. For scFvs that bind to antigens overexpressed on CLL cells, the CLL/PBMC ratio of fluorescent intensities is >1.0.

Fig 11. Comparison of scFv-9 antigen and CD38 expression on CLL cells.

Patient ID	% CD19+	% CD38+	% scFv-9+	ScFv-9 Level	CD38	ScFv-9
ML	80	40	98	266	Hi	Hi
IB	86	87	96	366	Hi	Hi
BH	76	56	86	284	Hi	Hi
JG	82	92	97	125	Hi	Lo
RE	87	97	100	125	Hi	Lo
EM	91	8	95	268	Lo	Hi
HS	76	11	94	268	Lo	Hi
MP	40	6	95	280	Lo	Hi
JR	81	12	92	124	Lo	Lo
GB	65	20	98	187	Lo	Lo

Fig 12. Identification of scFv Antigens

Cell-surface biotinylation (CLL-AAT cells)

Membrane isolation (nitrogen cavitation, differential centrifugation)

Immunoprecipitation with scFv-HA coupled to Anti-HA beads

- SDS-PAGE

Detection by Coomassie-stain or AP-streptavidin Western blot

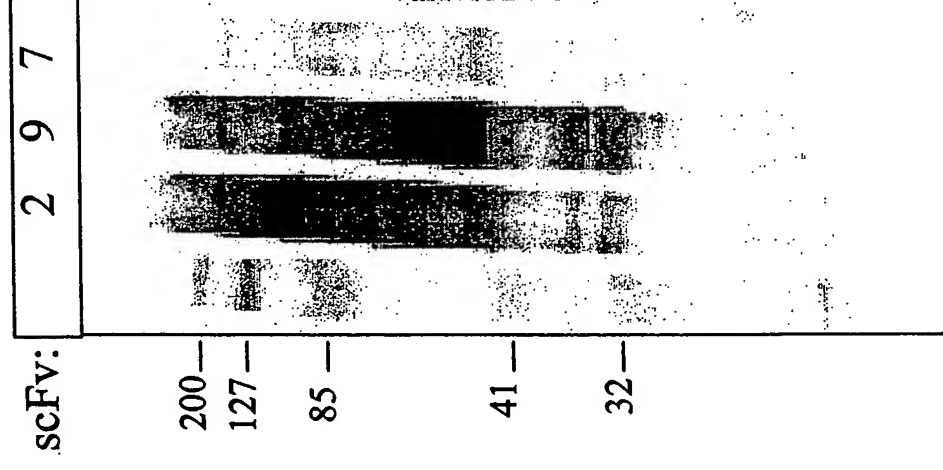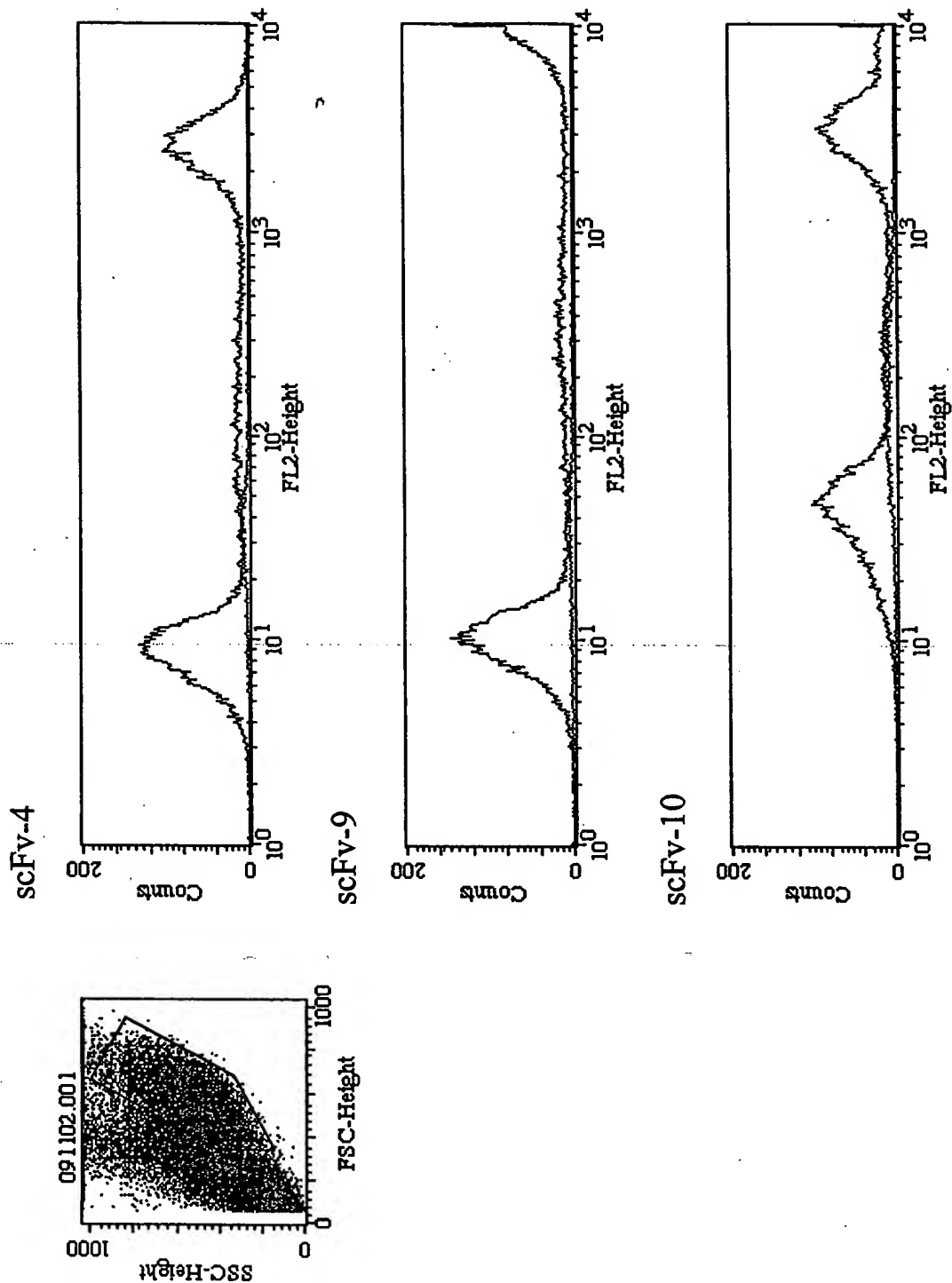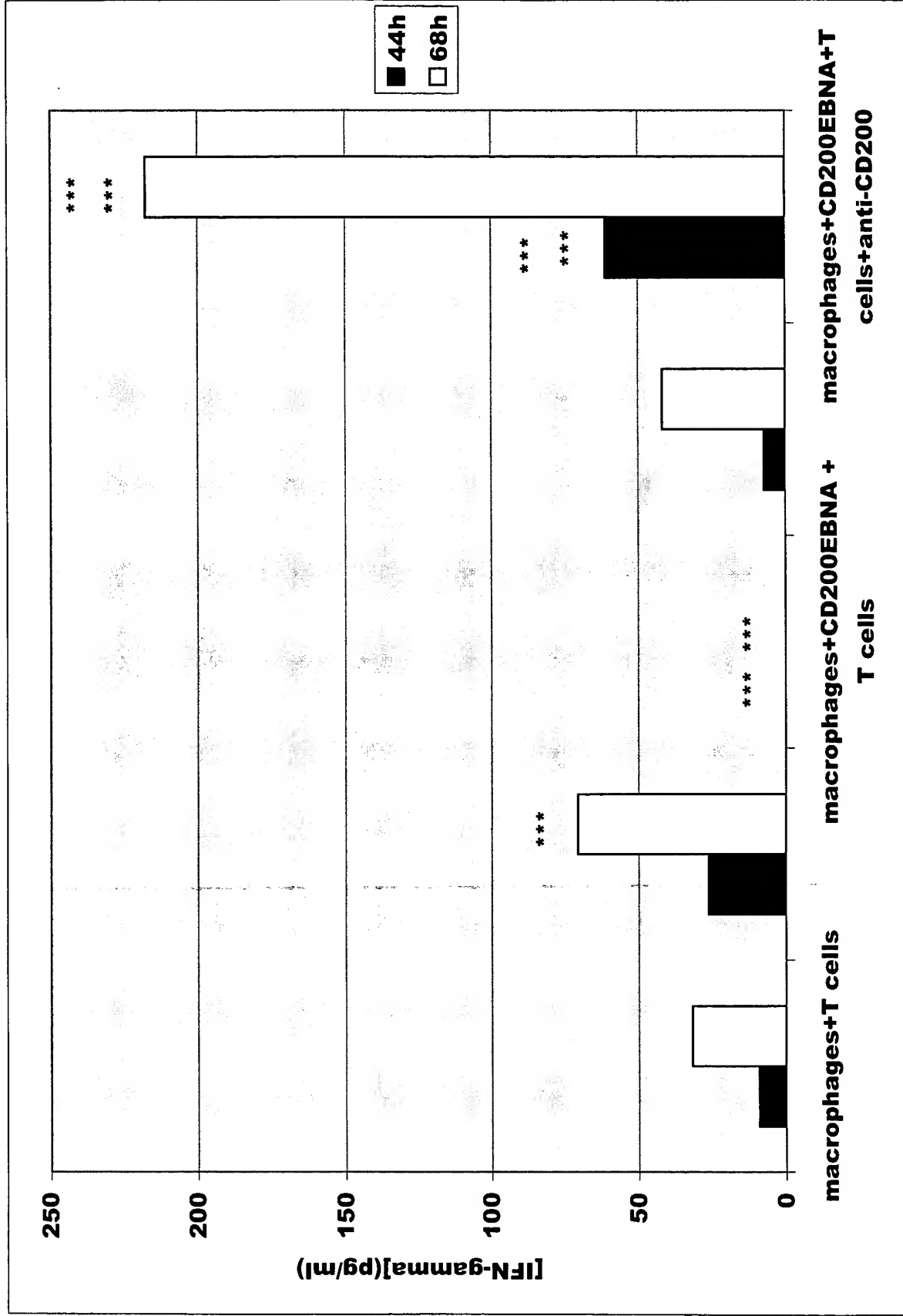- MALDI-MS or LC-MS/MS to obtain peptide mass spectra/peptide sequences

scFv: 2 9 7

200 —
127 —
85 —
41 —
32 —

Figure 13

Figure 14

Figure 15

Figure 16